et al., 2007). IFN-β signaling, in turn, suppresses chemokine secretion by microglia, and this suppression decreases infiltration by peripheral immune cells. In addition, IFN-β decreases the uptake and presentation of other nervous tissue antigens and inhibits the amplification of inflammation via epitope spreading.

Recently, in another publication, Guo et al. also examined the role of type 1 IFN signaling in EAE (Guo et al., 2008). They too discovered that IFNAR-deficient mice had defects in innate immune cell function, but they report a striking difference between those reported by Prinz et al. (2008) on the effects of type 1 IFN on the development of T helper 17 (Th17) cells. Prinz et al. (2008) observed no effect on the development of Th17 cells or expression of cytokines involved in their function. In contrast, Guo et al. (2008) demonstrated that the immunosuppressive effect of type 1 IFN is due to the downregulation of IL-23 and upregulation of IL-27, which is now known to inhibit Th17 cell differentiation. What could be the reason for these conflicting data?

On close inspection of the methods, there is a considerable difference in how each group induced disease. Prinz et al. (2008) used much less mycobacterium in the adjuvant than Guo (1 ug/ml versus 8 mg/ml). This difference could have a profound influence on the activation and cytokine production of the innate immune system and could be the cause of this important discordance in experimental outcomes.

IFN-β has been an exceptionally popular therapy for relapsing remitting MS. Because we now understand that IFN-β is a natural protector of brain tissue from inflammation, it is clear why exogenous administration of this cytokine has beneficial effects in diseases such as MS. However, not all patients respond to treatment. Therefore, defining the mechanisms responsible for the therapeutic effects of IFN-β has high relevance. Future studies, using both human and mouse models, must be designed to address what actually happens when IFN-β is administered as a therapy. Even though Prinz et al. (2008) do not elucidate the therapeutic mechanism of IFN-β, they describe an intriguing mechanism by which natural type 1 IFN expressed in the mouse suppresses inflammation and autoimmunity in the CNS. Prinz et al. (2008) have made an important discovery that provides key information for the community of scientists and physicians interested in demyelinating diseases such as MS and also for immunologists interested in autoimmunity in general.

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Taking a Toll Road to Better Vaccines

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Effective subunit vaccines must elicit strong CD4$^+$ T cell responses. In this issue of *Immunity*, Malherbe et al. (2008) find that the ability of adjuvants to stimulate high-avidity T cell responses correlates with Toll-like-receptor engagement.

Charlie Janeway referred to adjuvants as "the immunologist's dirty little secret," but they still offer the best hope for establishing safer and more effective subunit vaccines (Janeway, 1989). Adjuvants are nonspecific stimulators of the immune system, and many are thought to operate through activation of Toll-like receptors on antigen-presenting cells (McKee et al., 2007). However, the molecular mechanisms underlying adjuvant effects have not been well defined. To design adjuvants best suited to improve vaccine immunogenicity without increasing unwanted side effects, we must first delineate the properties that define good adjuvants and then elucidate their molecular effects. The effect of adjuvants on T cell responses has, until

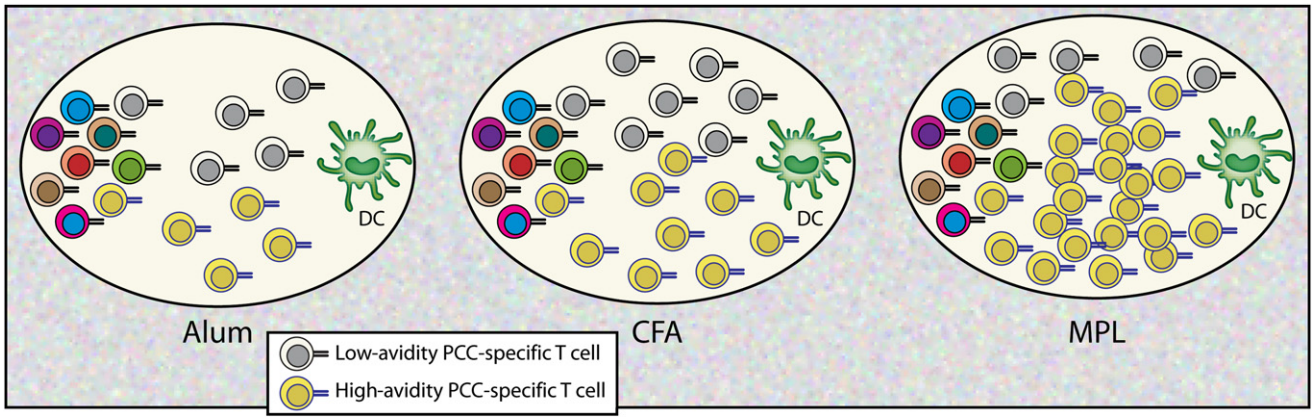

Figure 1. Adjuvants Regulate CD4$^+$ T Cell Responses
The number and avidity of antigen-specific T cells that develop in response to protein antigen varies with adjuvant. The depot-forming, non-TLR-ligand adjuvant alum elicits a small number of clonal PCC-specific T cells with relatively low-avidity TCRs. The depot-forming, multiple-TLR-ligand-containing adjuvant CFA induces a larger number of clonal PCC-specific T cells with higher-avidity TCRs. The adjuvant that induced the highest number of PCC-specific T cells with the highest avidity TCRs was MPL, a dispersable adjuvant that specifically stimulates TLR4.

now, been relatively unexplored. In this issue of *Immunity*, Malherbe et al. (2008) show that adjuvants have a differential ability to stimulate high-avidity CD4$^+$ T cells.

Effective and safe vaccines represent one of the most important medical advances in human history. The scourge of smallpox has been eradicated globally, and the devastation of paralytic poliomyelitis has been absent from the Americas since 1991. Despite these historic successes, many challenges in vaccine development and use remain. To be effective, a vaccine not only must stimulate the appropriate immunologic response for pathogen neutralization, such as antibody production or cytotoxic T cell generation, but also must promote long-lived immunity via development of memory T cells. To be safe, a vaccine must avoid generating severe systemic inflammation when administered. A perfect vaccine therefore achieves robust and appropriate immunogenicity without undue inflammation. Unfortunately, no vaccine is perfect. Live attenuated viruses, still used to immunize against measles, mumps, rubella, and varicella, are safe for the majority of recipients. However, the varicella vaccine can cause severe disease if inadvertently given to a child with an unrecognized immunodeficiency (Gershon, 2003). Killed or inactivated organisms are used for immunization of diseases such as influenza and polio, but inclusion of whole bacterial products can produce severe systemic side effects. Prior to the introduction of the acellular pertussis vaccine, use of whole-cell pertussis was associated with a relatively high frequency of adverse events such as high fevers, seizures, and encephalopathy (Decker and Edwards, 2000). Subunit vaccines avoid the side effects of whole-cell or killed organisms and are safe for immunocompromised individuals, but they can have decreased immunogenicity. For example, the polysaccharide antigens of the pneumococcal vaccine must be conjugated to a protein carrier in order to elicit a protective immune response in children under two years of age, those most at risk for severe pneumococcal infection. Furthermore, use of the above strategies has not yet enabled the development of successful vaccines against some of the biggest killers, such as malaria and HIV.

The absence of data regarding adjuvant effects on T cells is due in part to the lack of intermediate biomarkers for T cell responses. Although antibodies can be followed easily in the serum, T cell responses are more difficult to assess. Despite the recent advances in understanding T cell recognition of antigen, we still do not know precisely what features of the TCR:pMHC interaction are desirable in a vaccine. Cases have been made for the measurements of affinity and kinetics such as K_D, K_{off}, or two-dimensional K_D (Qi et al., 2006; Williams et al., 1999), but no single parameter completely correlates with the sensitivity of the T cell. Furthermore, the effects of different adjuvants on these parameters have not been determined.

Here, Malherbe et al. (2008) use the response of naive T cells to pigeon cytochrome C (PCC) to determine adjuvant effects on naive T cell responses. This system enables the analysis of antigen-specific responses in a normal (non-TCR-transgenic) mouse. T cells specific for PCC 94-103-I-Ek epitope fortuitously utilize conserved Vα11 and Vβ3 TCR chains. McHeyzer-Williams and Davis (1995) exploited this feature of the PCC response previously, showing that PCC specific T cells could be purified from a normal mouse through the use of monoclonal antibodies and then of single-cell PCR to analyze the TCR chains. The invention of MHC tetramers has greatly expanded the ability of antigen-specific T cells to be purified and the relative avidity of the TCRs to be determined (Altman et al., 1996; Moon et al., 2007). The McHeyzer-Williams laboratory has established a set of eight preferred features within the CDR3 of the expressed TCR$\alpha\beta$ chains in PCC-specific T cells, with which they can identify the responding clones and categorize their avidity.

Malherbe et al. (2008) have now characterized and compared the primary PCC-specific T cell responses stimulated in the presence of different adjuvants. They tested five adjuvants: three depot-forming (alum, CFA, IFA) and two dispersible formulations (CpG and MPL). These adjuvants also differed in their ability to engage Toll-like receptors. Alum and IFA contain no TLR ligand, whereas CFA engages multiple TLRs, CpG engages TLR9, and MPL engages TLR4. All adjuvants stimulated PCC-specific T cells

but differed in the number of antigen-specific T cells generated (Figure 1). Alum induced the fewest number of T cells, whereas MPL induced the most. Somewhat surprisingly, all adjuvants, regardless of TLR ligation, were able to induce the clonal expansion of PCC-specific T cells. However, important differences between adjuvants were revealed when the avidity of the expanded T cells was characterized by either the number of preferred features of the CDR3 or tetramer binding. Alum, IFA, and CFA promoted PCC-specific T cell clones that were of lower avidity, whereas CpG and MPL preferentially stimulated the higher avidity T cell clones (Figure 1). Thus, the inclusion of TLR agonists into the adjuvant formulations directly correlated with increased accumulation of higher-avidity T cells. Interestingly, the clonal selection of PCC-specific T cells was independent of antigen dose. This implies that there does not appear to be interclonal competition among $CD4^+$ T cells for antigen, in contrast to what has been shown for $CD8^+$ T cells.

In summary, the system described by Malherbe et al. (2008) represents an important step forward in quantifying the parameters of T cell responses that may correlate with vaccine efficacy. It will now be critical to determine whether these five adjuvants elicit similar responses with different model antigens. With the clear demonstration that adjuvant formulation can substantially modify the number and avidity of antigen-specific T cells selected in response to soluble-protein immunization, we can investigate the effect of these different populations of T cells on the development of T cell memory and effective pathogen responses. For instance, this system could be employed to determine whether higher-avidity T cells result in improved development of T cell memory. Furthermore, this system could be exploited to clarify mechanisms by which adjuvants operate. Open questions include whether adjuvants are acting upon T cells, antigen-presenting cells, or both. Also, although this study showed that adjuvants that are TLR ligands induced higher-avidity T cell responses, it is not yet clear that TLR-agonist adjuvants will markedly improve vaccine efficacy. However, the findings of Malherbe et al. (2008) represent one more step along the road toward the development of effective subunit-based vaccines.

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The Immunostimulatory Power of Acute Viral Infection

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In this issue of *Immunity*, Miller et al. (2008) use multiple independent techniques to demonstrate that antiviral T cell responses after acute human infection are much larger than previously realized.

Several groups have demonstrated that antiviral $CD8^+$ T cell responses in mice can be enormous—with virus-specific T cells representing up to 50%–80% of the total $CD8^+$ T cell population at the peak of the immune response and/or the anatomical site of infection (Murali-Krishna et al., 1998). Such dramatic T cell responses were thought to be associated with only a few select pathogens (e.g., lymphocytic choriomeningitis virus; LCMV) or perhaps related to the large virus doses and invasive routes of infection (e.g., intraperitoneal administration) that are often used during experimental infection of animals. Acute viral infection of humans, on the other hand, was thought to result in a much smaller $CD8^+$ T cell response. However, as described in this issue of *Immunity*, Miller et al. (2008) provide compelling evidence indicating that the magnitude of $CD8^+$ T cell responses identified in humans after acute viral infection might indeed rival the magnitude of antiviral T cell responses observed in experimental animal models.

Miller et al. (2008) analyzed virus-specific $CD8^+$ T cell responses against two unrelated viruses: yellow fever virus (YFV) and vaccinia virus (VV). Although both YFV and VV represent acute viral infections, they differ in many ways. YFV is a small RNA virus that encodes just ten genes, and following subcutaneous inoculation, it spreads systemically, resulting inn a transient viremia in the infected